For Research Use Only *Not for Diagnostic Use*

HDV Ag

Catalog #: WD6396

HEPATITIS D VIRUS ANTIGEN ELISA KIT
Two-Step Incubation, Double Antibody Sandwhich Principle

INSTRUCTIONS FOR USE

This kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antigens to hepatitis D virus (HDV-Ag) in human serum or plasma. For Research Use Only

SUMMARY

Hepatitis D is caused by Hepatitis D virus (Delta agent) – a defective (36nm-43nm) enveloped RNA virus, which requires co-infection with Hepatitis B virus (HBV) for its replication. Transmitted percutaneously or sexually through contact with infected blood or blood products, HDV is associated with the most severe forms of chronic and acute hepatitis in many Hepatitis B -HBsAg positive patients. Since the infection with HDV requires infection with HBV, the development of the disease depends on whether the two viruses infect simultaneously (coinfection) or whether the newly infected HDV patient is also a chronic HBV carrier (superinfection). The co-infection with HDV can lead to severe acute hepatitis disease with low risk of chronic stage development. Chronic HBV carrier patients superinfected with HDV are at risk to develop chronic HDV disease, which can lead to cirrhosis in 70%-80% of the patients. The serological diagnosis of HDV is based on detection of specific HDV antibodies (anti-HDV) or antigens (HDV-Ag). During HBV-HDV coinfection, detectable concentrations of HDV specific antigens first appear six to eight week after exposure and clear during convalescence together with elevated levels of HDV antibodies which indicate recovery. The circulating HDV-Ag is indicative of an acute infection. It is typically present only transiently in very low levels. During HDV super infection, detectable levels of HDV antigens appear two weeks after exposure and failure to clear indicates possible progression to long term, chronic carrier stage.

PRINCIPLE OF THE ASSAY

This HDV-Ag ELISA is based on solid phase, two-step incubation Double Antibody Sandwich Principle. Polystyrene microwell strips are pre-coated with purified antibodies specific to HDV. Patient's serum/plasma is added together with Extraction Solution. If present, the viral particles are disrupted and the specific HDV antigens are captured in the wells. The microwells are washed to remove unbound serum proteins. A second antibody conjugated with Horseradish Peroxidase (HRP) is added and during the second incubation, this antibody will be bound with the captured antigen. After washing to remove unbound conjugates, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HDV antigens remain colorless.

COMPONENTS

96 Tests

- **MICROWELL PLATE** 1 plate

Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. **8×12/12×8-well strips** per plate. Each well contains purified antibodies reactive to HDV. The microwell strips can be broken to be used separately. Place unused wells in the plastic sealable storage bag together with the desiccant and return to 2~8ºC.

- **NEGATIVE CONTROL** 1 vial

Yellowish liquid filled in a vial with green screw cap. 0.5 ml per vial. Protein-stabilized buffer tested non-reactive for HDV-Ag. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **POSITIVE CONTROL** 1 vial

Red liquid filled in a vial with red screw cap. 0.5 ml per vial. Purified HDV-Ag diluted in protein-stabilized buffer. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **HRP-CONJUGATE REAGENT** 1 vial

Red liquid in a white vial with red screw cap. 12 ml per vial. Horseradish peroxidase-conjugated anti-HDV antibodies. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **EXTRACTION SOLUTION** 1 vial

Blue solution filled in a white vial with black screw cap. 6ml per vial. Chaotropic agent for disruption of the viruses during incubation. Once open, stable for one month at 2-8ºC.

- **STOCK WASH BUFFER** 1 bottle

Colorless liquid. 50ml per bottle. pH 7.4 20× PBS (Containing Tween-20 as a detergent). **DILUTE BEFORE USE** The concentrate must be diluted 1 to 20 with distilled/deionized water before use. Once diluted, stable for one week at room temperature or for two weeks when stored at 2-8ºC.

- **CHROMOGEN SOLUTION A** 1 vial

Colorless liquid filled in a white vial with green screw cap. 7 ml per vial. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **CHROMOGEN SOLUTION B** 1 vial

Colorless liquid filled in a black vial with black screw cap.

TMB solution. Tetramethylbenzidine dissolved in citric acid. 7 ml per vial. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **STOP SOLUTION** 1 vial

Colorless liquid filled in a white vial with white screw cap. 7 ml per vial. Diluted sulfuric acid solution (2.0M H_2SO_4). Ready to use as supplied.

- **PLATIC SEALABLE BAG** 1 unit

For enclosing the strips not in use.

- **CARDBOARD PLATE COVER** 2 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

PACKAGE INSERTS 1 copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5ºC.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

SPECIMEN COLLECTION，TRANSPORTATION AND STORAGE

1. **Sample Collection**: Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they can give false results in the assay. Do not heat inactivateD samples. This can cause sample deterioraration.
2. **Transportation and Storage:** Store samples at 2-8ºC. Samples not required for assaying within 3 days should be stored frozen (-20ºC or lower). Avoid multiple freeze-thaw cycles.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.

The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 ºC, **do not freeze**. To assure maximum performance of this HDV-Ag ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR RESEARCH USE ONLY**

The ELISA assay is time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and samples to reach room temperature (18-30ºC) before use. Shake reagent gently before use. Return at 2-8ºC immediately after use.
4. Does not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
5. When reading the results, ensure that the plate

bottom is dry and there are no air-bubbles inside the wells.

6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all the wells.
8. Calibrate the pipette frequently to assure the accuracy. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations. Never pipette solutions by mouth.
9. The use of automatic pipettes and disposable tips is recommended.
10. Assure that the incubation temperature is 37ºC inside the incubator.
11. When adding samples avoid touching the well's bottom with the pipette tip.
12. When measuring with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
13. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
14. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121ºC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.
15. The Stop Solution contains 2M H_2SO_4. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
16. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.

ASSAY PROCEDURE

Step 1 **Reagents preparation:** Allow the reagents to reach room temperature **(18-30°C).** Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step 2 **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank (**e.g. A1**, neither samples nor HRP-Conjugate should be added into the Blank well). Use only number of strips required for the test.

Step 3 **Adding Sample:** Add **50 µl** of Positive control, Negative control, and specimen into their respective wells. Add **50µl** of Extraction Solution supplied with the kit to each well. Then mix slightly. Note**: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control and Extraction Solution as to avoid cross-contamination. Never add Extraction Solution to the Blank Well.**

Step 4 **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37**°C. It is recommended to use water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step 5 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the strips plate onto blotting paper or clean towel, and tap out any remainders.

Step 6 **Adding HRP-Conjugate:** Add **100 µl** of HRP-Conjugate Reagent into each well except the blank, and mix gently. **Never add HRP-Conjugate to the Blank Well.**

Step 7 **Incubating :**Cover the plate with the plate cover and incubate the plate for **30 minutes at 37°C.**

Step 8 **Washing**: Wash the plate **5 times** with wash buffer as **step 5.**

Step 9 **Coloring:** Dispense **50 µl** of Chromogen A and **50 µl** Chromogen B solution into each well including the **Blank** and mix by tapping the plate gently. Incubate the plate at **37°C for 10 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HDV-Ag Positive sample wells.

Step 10 **Stopping Reaction:** Using a multichannel pipette or manually add **50 µl** Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HDV-Ag Positive sample wells.

Step 11 **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **5** minutes after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is

based on Dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. **Calculation of Cut-off value (C.O.) = *NC × 2.1**

*NC = the mean absorbance value for three negative controls.)

Important: If the mean OD value of the negative control is lower than 0.05, take it as 0.05.

Example:

1. Calculation of NC:

Well No	B1	C1	D1
Negative controls OD values	0.020	0.012	0.016

NC=0.016 (Nc is lower than take 0.05 so take it as 0.05)

2. Calculation of Cut-off value (C.O.)=0.05x2.1=0.105

If one of the Negative Control values does not meet the Quality control range specifications, it should be discarded, and the mean value is calculated again using the remaining two values.

If more than one control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. **Quality control range**
   1. The absorbance of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
   2. The absorbance value OD of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.
   3. The absorbance value OD of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1)□Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis D virus specific antigens have been detected with this HDV–Ag ELISA kit. **Positive Results**:(**S/C.O.≥1)**□Samples giving an absorbance greater than or equal to the Cut-off value are considered initialy reactive ,which indicates that HDV virus specific antigens have been detected using this HDV–Ag ELISA kit. Any initially reactive samples must be retested in duplicates. Repeatedly reactive samples can be considered positive for antigens to HDV

Borderline (S/CO =0.9-1.1) : Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline. Retesting of these samples in duplicates is recommended. Repeatedly reactive samples could be considered positive for antigens to HDV.

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is designed to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HDV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
3. The prevalence of the marker will affect the assay's predictive values.

VALIDITY

Please do not use this kit beyond the expiration indicated on the kit box and reagent labels

REFERENCES:

1. Purcell RH and Gerin JL, Hepatitis Delta virus. In: Fields Virology,3rd ed. Philadelphia,Lippincott-Raven,1996.
2. Hadziyannis SJ. Hepatitis delta: an overview. In: Rizzetto M, Purcel RH, Gerin JL, and Verme G,eds. Viral hepatitis and liver disease, Turin, Edizoni Minerva medica, 1997
3. Lai MCC. The molecular biology of hepatitis Delta virus. Annual Review of Biochemistry, 1995 64:259-286
4. Centers for Disease Control and Prevention. Epidemiology and Prevention of Viral Hepatitis A to E: An Overview 2000.
5. Hepatitis Delta: WHO/CDS/CSR/NCS 2001.1

Express Biotech International
P.O. BOX 458
Thurmont, MD 21788 USA
Tel: 301-228-2444 Fax: 301-560-6570
Toll Free: 888-562-8914
www.xpressbio.com **info@xpressbio.com**

XpressBio
Life Science Products